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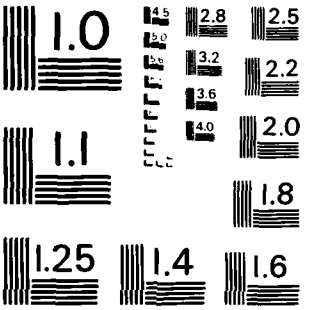

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The mechanism by which oxygen causes convulsions is not clear. Although many biochemical changes in brain have been reported to occur as a result of oxygen exposure, these changes are not necessarily the cause of oxygen-induced convulsions. From in vivo studies in mice, inhibition of brain energy metabolism was not found. Furthermore, although oxygen caused an increased oxidation of pyridine nucleotides, the decrease in both NADPH and NADH in cerebral cortex was not related to the susceptibility of mice to oxygen convulsions. GABA in brain cortex was decreased by oxygen, however, this decrease did not influence the sus-

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ceptibility of mice to oxygen convulsions. The most effective CNS protectants seem to be those agents containing a disulfide bridge in their molecular structure, and which can be reduced to thiols. Since thiols are good free radical scavengers, free radicals may be the initial event in provoking the insult to produce oxygen convulsions.

Final Report

A number of studies were proposed to be carried out, all designed to more clearly investigate the mechanism(s) of central and pulmonary oxygen toxicity. In addition, disulfiram was used as a pharmacological tool to better understand the mechanism of CNS oxygen toxicity. In order to use disulfiram, studies also were proposed to develop the analytical methods so that the elimination characteristics of disulfiram could be better understood.

Summary of Results Obtained

Neither oxygen at high pressure (OHP) nor disulfiram alone significantly altered cerebral ATP in mice prior to the onset of convulsions. However, the combination of OHP and disulfiram elevated cerebral ATP with the increase most prominent during the initial period of the OHP exposure. OHP alone had no effect on cerebral phosphocreatine. Disulfiram, both alone and in combination with OHP elevated phosphocreatine. No significant differences in cerebral cortical ATP or phosphocreatine were found between controls and mice sacrificed either at the onset or nine seconds after the onset of convulsions.

The concentration of lactate and pyruvate increased as the length of time the mice were exposed to OHP increased. Disulfiram which by itself lowered cerebral lactate, prevented the increase in lactate and pyruvate in mice exposed to OHP. As found with ATP and phosphocreatine, lactate and pyruvate did not differ significantly from controls at either the onset or nine seconds after the onset of oxygen-induced convulsions.

Both disulfiram and OHP increased cerebral glucose. The effect of disulfiram and OHP were somewhat additive as the mean for the combination treatment was significantly higher than that of the animals receiving OHP alone. Glucose remained elevated at both the onset and nine seconds after the onset of oxygen convulsions.

Studies were also carried out to investigate the role of glutamate, GABA, and ammonia in brain on oxygen-induced convulsions. OHP was not found to affect the concentration of either glutamate or ammonia in the cerebral cortex prior to the onset of convulsions. Only after the animals had convulsed were cerebral ammonia levels increased. The concentration of GABA in the cerebral cortex was lowered by OHP. Pretreatment with disulfiram prevented the onset of oxygen convulsions and lung damage, but did not prevent the lowering of brain GABA.

Studies were carried out to assess the contribution of alterations in blood glucose to the increase in brain glucose produced by OHP. The results of these studies indicate that OHP elevated both brain and blood glucose. Both the tendency of the blood/brain glucose ratios (not significant) to increase with longer oxygen exposures and the fact that blood glucose levels were increased while brain glucose (hyperactive group) were not yet statistically different from controls suggest that the increased brain glucose followed and was probably a result of an increase in blood glucose.

Adrenalectomy blocked the increase in blood glucose and greatly reduced

the increase in brain glucose observed in normal animals. This suggests that a large portion of the increased brain glucose observed in normal animals exposed to OHP can be attributed to a peripheral response to the stress of OHP and the resulting increase in blood glucose. There are several reasons why adrenalectomy failed to completly prevent the increase of brain glucose. Oxygen may have inhibited glycolysis, or the decompression prior to sacrifice may have altered the uptake of glucose into the brain.

The results suggest that OHP-induced convulsions are not due to either a fall in brain energy reserves or an inhibition of brain energy metabolism. Although OHP increased brain lactate and glucose levels, the increased glucose was found to be the result of a stress effect and adrenal cortex activation. However, neither the increased glucose or lactate levels seem to be involved in the genesis of oxygen-induced convulsions. In conclusion, it can be stated that oxygen convulsions do not appear to be the result of simple derangement in brain energy metabolism.

Ammonia

The results of several studies indicate that neither OHP nor disulfiram alone or in combination had any effect on cerebral ammonia. However, because it is possible that oxygen may cause a precipitous change in brain ammonia just preceding the convulsion which would not be detected, the effect of OHP on brain ammonia in mice during hyperactivity, 100% of the CT_{50} (time taken for 50% of the mice to convulse), and at the onset of convulsions was determined.

The results of these studies demonstrated that prior to the onset of convulsions OHP had no effect on cerebral ammonia. Seizures, whether induced by oxygen or pentylenetetrazole, result in a significant increase in brain ammonia. These data suggest that oxygen-induced seizures are not the result of alterations in brain ammonia. Although cerebral ammonia was elevated at the "onset" of oxygen-induced seizures, this was felt to be a result rather than a cause of the increased neuronal activity.

Pentylenetetrazole apparently caused a greater increase in ammonia than OHP. However, this is believed to be an artifact resulting from the method of sacrifice. The pentylenetetrazole-convulsed mice were kept in individual cages and had to be caught and removed before they could be frozen. The OHP convulsed mice were on a platform containing a trap door which opened directly over a flask of liquid nitrogen and thus could be frozen in liquid nitrogen much faster than the pentylenetetrazole-treated mice.

No changes in ATP or phosphocreatine were found in OHP convulsed animals. However, ammonia was increased in these OHP convulsed animals. Although the significance of this is uncertain, it suggests that the increase in ammonia is not an indication of an impending energy deficit.

Glutamate

The results of the studies indicated that neither OHP nor disulfiram alone or in combination had any effect on cerebral glutamate. However, because OHP may cause a precipitous change in brain glutamate just prior to

the convulsion which would not be detected in this study, the effects of longer oxygen exposures on brain glutamate were investigated. The studies indicated that OHP has no effect on cerebral glutamate either prior to or at the onset of convulsions. The data thus suggest that oxygen convulsions are not the result of a change in total glutamate levels. That the oxygen treatments employed altered the level of two substances, GABA and ammonia, metabolically linked to glutamate but did not alter glutamate levels is not too surprising. Glutamate is present in much higher concentrations than either GABA or ammonia, thus glutamate changes equal in magnitude to those observed with GABA and ammonia would not be detected. Also, in addition to the reactions involved in GABA formation and ammonia detoxification, glutamate participates and in turn its levels are regulated by numerous other reactions.

As a result of some earlier preliminary studies, it appeared that oxygen protective compounds function either as antioxidants, or because of their molecular architecture, excellent free radical scavengers. A series of studies were also carried out to determine the mechanism by which disulfiram provides its protective action. It appears that the disulfide bridge is an important component of the disulfiram molecule. Removal of the disulfide bridge produces a loss in oxygen protective action. Disulfiram is reduced *in vivo* to its corresponding thiol, diethyldithiocarbamate. Since thiols are well known free radical scavengers, it appears that disulfiram may provide its protective action by acting as an antioxidant, possibly as a free radical scavenger. Tetraethylthiuram disulfide (disulfiram) has been shown to be an effective oxygen protectant. Other disulfides such as methylene bis (NN diethyldithiocarbamate); tetramethylthiuram disulfide; bis (4-methyl-1-homopiperazinylthiocarbonyl) disulfide; cystine; homocystine; cystamine; oxidized glutathione; 3-carboxypropyl disulfide; 2,2' and 4,4' dithiodipyridine also protected against oxygen toxicity (6 ATA) when administered ip to mice. The corresponding thiol of these disulfides (diethyldithiocarbamate; cysteine; homocysteine; cysteamine; reduced glutathione) and mercaptosuccinic acid also protected mice from oxygen toxicity. Optimum protection from oxygen toxicity with disulfides necessitated longer pretreatment times than the corresponding thiols. This suggests that disulfides, in order to act as oxygen protectants, must be reduced to the thiol. Since thiols are excellent free radical scavengers, it is proposed that these agents protect against oxygen toxicity by acting as antioxidants. Disulfiram, and other disulfides may therefore act as pro-drugs, with the thiol being the active moiety. Other disulfides and thiols, depending on their physico/chemical properties would also be expected to act in a similar manner. Oxygen protectants which are neither disulfides nor thiols could act as antioxidants by some indirect manner, and may provide protection by a similar mechanism.

A comparative effectiveness of disulfiram with vitamin E, GABA, and reduced glutathione was carried out. It was found that disulfiram, GABA, and reduced glutathione all appeared to be equally effective in protecting against oxygen convulsions. Vitamin E was ineffective in protecting against oxygen convulsions.

Convulsive studies also were carried out to compare the oxygen protecting action of disulfiram with succinate. In these studies it was found that only high doses of succinate (12 mM/kg) provided protection from

oxygen convulsions. It was also found that succinate provided protection only when animals were pretreated one hour before oxygen exposure. Animals pretreated either 30 minutes or 2 hours before oxygen exposure were not protected from oxygen convulsions.

Histopathological studies were carried out to determine the comparative effectiveness of disulfiram, vitamin E, and GABA and reduced glutathione in protecting against pulmonary oxygen toxicity. Mice were given glutathione, 12 mM/kg ip, 15 minutes before oxygen exposure; GABA, 20 mM/kgip, 15 minutes before oxygen exposure; and vitamin E, 0.5 mg/kg, 18 hours before oxygen exposure.

The results from these studies indicate that glutathione was not as effective as disulfiram in protecting against oxygen-induced lung damage. Both vitamin E and GABA in the doses employed appeared to be effective as disulfiram in protecting against pulmonary oxygen toxicity. As an overall protective agent against both convulsions and lung damage, disulfiram appears to be superior to glutathione, vitamin E, and GABA. Studies comparing succinate with disulfiram in protecting against lung damage have not yet been carried out.

$NADP^+$, NADPH and glucose 6-phosphate dehydrogenase were determined in the cerebral cortex of mice exposed to high O_2 pressure for 0, 8 and 16 min. These time intervals corresponded to 0, 50 and 100% of the CT_{50} (the time taken for 50% of the mice to convulse). Cerebral $NADP^+$, NADPH and glucose 6-phosphate dehydrogenase also were determined in O_2-exposed mice exhibiting hyperactivity, convulsions, and in mice killed 10 seconds after convulsions. Similar increases in cortical $NADP^+$ and decreases in NADPH were found in mice exposed to 6 atm of 100% O_2 for 0, 50 and 100% of the CT_{50}, during hyperactivity, onset of seizure and 10 seconds after convulsions. The $NADP^+$/NADPH ratio increased approximately 25% at 0% of the CT_{50}, and remained at this increased value at all O_2-exposure periods including the hyperacive state, onset of seizure and 10 seconds after convulsions. Identical changes in cerebral $NADP^+$, NADPH and the $NADP^+$/NADPH ratio were found in mice exposed for 16 min to 100% O_2 at 1, 3.5 or 6 atm. No change in cerebral glucose 6-phosphate dehydrogenase was found in mice exposed to 6 atm of 100% O_2 during the various stages of O_2 toxicity. Only in the 10 second post-convulsive group was a statistically significant decrease in glucose 6-phosphate dehydrogenase observed. Disulfiram [bis(diethylthiocarbamoyl)disulphide], an effective O_2-protective agent, did not prevent the O_2-induced increase in cerebral $NADP^+$ and the $NADP^+$/NADPH ratio, or decrease in NADPH.

Experiments were done <u>in vivo</u> to determine the effect of oxygen at high pressure (OHP) on NAD^+ and NADH in mouse brain cortex. A 7% decrease (N.S.) in NADH was found in brain cortex from mice exposed to 6 atm of 100% oxygen for 8 min, while a 20% decrease ($P<0.01$) in cortical NADH, when compared to controls, occurred when mice were exposed to this oxygen pressure for either 16 min or 48 min. A 20% decrease ($P<0.05$) in cortical NADH was also observed in mice which had been killed during hyperactivity (a state preceding convulsions), at seizure onset, or 10 seconds post-convulsions. No measurable change in cortical NAD^+ was observed at any of these oxygen exposure times or stages of toxicity. When mice were exposed to either 3.5 atm or 6 atm of oxygen for 16 min, a statistically

significant decrease in cortical NADH ($P<0.01$) coupled with an increase in the $NAD^+/NADH$ ratio was found only at 3.5 atm and 6 atm, and not at 1 atm. The decrease in cortical NADH and increase in the $NAD^+/NADH$ ratio were reversed when mice were decompressed and exposed to air for 30 min. Disulfiram, a drug found to delay the onset of oxygen seizures, did not prevent the oxygen-induced decrease in cerebral NADH or increase in the $NAD^+/NADH$ ratio. The decrease in cortical NADH in mice exposed to OHP did not correlate with the onset of oxygen-induced convulsions.

Mice were exposed to 6 atm of 100% O_2 and killed at the onset of hyperactivity, convulsions and 10 seconds post convulsions. Examination of brain cortex from mice killed at these stages of O_2 toxicity revealed no change in oxidized glutathione (GSSG), non-protein sulfhydryls (NPSH), total glutathione (GSH + GSSG), the GSH/GSSG ratio, glutathione reductase and glutathione peroxidase. Mice exposed to 4 atm for 1 hour or 6 atm for 16 min exhibited a 36% and 33% decrease in lung NPSH respectively, but no change in cortical NPSH was observed. Although intraventricular diethylmaleate (DEM) decreased cerebral NPSH 72%, no change in the susceptibility of mice to O_2 convulsions was found. Disulfiram, an effective O_2 convulsive protectant had no effect on either cortical NPSH or total glutathione.

The relationship betwen the concentration of unsaturated lipid, free radical initiator, and oxygen concentration on the kinetics of lipid peroxidation was determined. The rate of lipid peroxidation was studied with the thiobarbituric acid (TBA), diene conjugation (DC), and ferrithiocyanate (Fe-SCN) methods. The rate of peroxidation was half-order with respect to unsaturated lipid, initiator, and oxygen. The half-order relationship could be expressed as: $\text{rate} = (fk_1k_2k_3/k_6^{1/2}(\text{azobisisobutyronitrile})^{1/2}(RH)^{1/2}(O_2)^{1/2}$. The half-order relationship was found with linoleic (18:2), linolenic (18:3), and arachidonic (20:4) acids. A linear relationship existed between the logarithm of unsaturation and the rate of peroxidation. No peroxidation of linolenic acid was indicated when the DC method was employed, but was when the TBA and Fe-SCN methods were used.

In summary, the mechanism by which oxygen causes convulsions is not clear. Although many biochemical changes in brain have been reported to occur as a result of oxygen exposure, these changes are not necessarily the cause of oxygen-induced convulsions. From *in vivo* studies in mice, inhibition of brain energy metabolism was not found. Furthermore, although oxygen causes an increased oxidation of pyridine nucleotides, the decrease in both NADPH and NADH in cerebral cortex was not related to the susceptibility of mice to oxygen convulsions. GABA in brain cortex was decreased by oxygen, however, this decrease did not influence the susceptibility of mice to oxygen convulsions. The most effective CNS protectants seem to be those agents containing a disulfide bridge in their molecular structure, and which can be reduced to thiols. Since thiols are good free radical scavengers, free radicals may be the initial event in provoking the insult to produce oxygen convulsions.

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